4th SCI/RSC Symposium on Anti-infectives Drug Discovery, Presentation #10

Inhibiting coronavirus 5' RNA capping with potent, selective, FIC small molecules

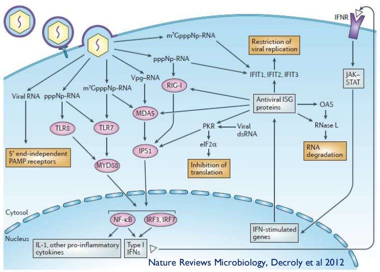
Angela Glen[1], Byron Andrews[1], Ralph Baric[2a], Elliot Bayle[1], Caitlin Duncan[1], Matthew Fyfe[1], Patrick Guest[1], Helen Harrison[1], Mark Heise[2b], Nat Moorman[2c], Oliver Rausch[1], Trevor Scobey[2a], Tim Sheahan[2a], Chris Swain[1], Sharon Taft-Benz[2b], Beth Thomas[1], Aleksandra Zemla-Brown[1]

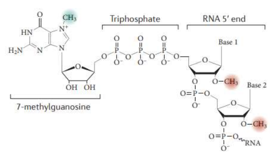
1) STORM Therapeutics Ltd, Babraham Research Campus, Cambridge CB22 3AT, United Kingdom
2) AViDD-READDI, Rapidly Emerging Antiviral Drug Development Initiative (READDI): a) Department of Epidemiology, UNC Chapel Hill, Gillings School of Public Health; b) Department of Genetics, UNC Chapel Hill, School of Medicine; c) Department of Microbiology and Immunology, UNC Chapel Hill, School of Medicine

Disclosure statement for presenting author: Angela Glen is a full-time employee of STORM Therapeutics Ltd, Cambridge (UK) angela.glen@stormtherapeutics.com

Introduction

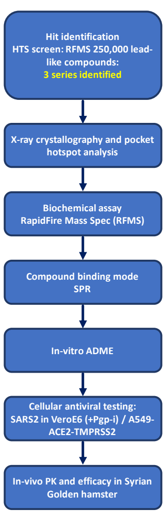
The RNA genome of SARS-CoV-2 contains a 5′ cap that facilitates the translation of viral proteins, protection from exonucleases and evasion of the host immune response. Some viruses, including coronaviruses, produce their own capping enzymes which include RNA methyltransferases. We describe the development and characterization of inhibitors of a coronavirus methyltransferase to prevent viral replication and to expose the virus to activate the host immune response.



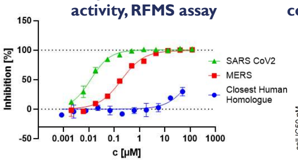


1) Identification of viral specific inhibitors

A Assay cascade

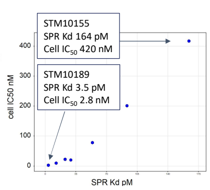


B Viral specific biochemical activity, RFMS assay

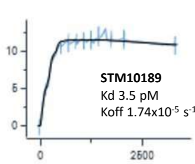


STM10155	SARS2	MERS	Hu Homologue
RFMS IC50 µM	0.012	0.19	111

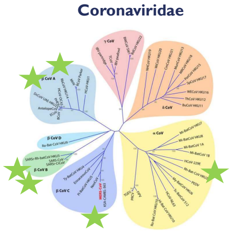
C Biochemical activity correlates with cell potency



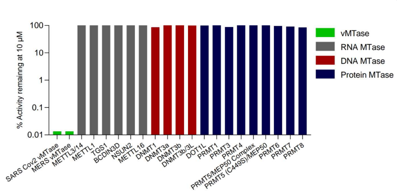
D Single digit picomolar binding by SPR



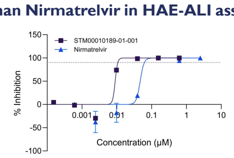
E Phylogenetic tree of Coronaviridae



F Methyltransferase selectivity panel



G STM10189 is 5-fold more potent than Nirmatrelvir in HAE-ALI assay

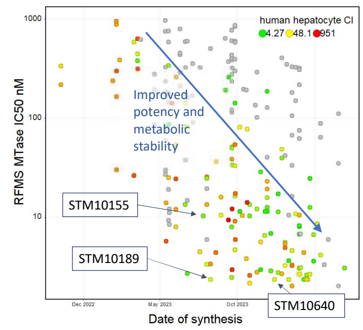


	STM00010189	Nirmatrelvir
IC90 (µM)	0.011	0.062

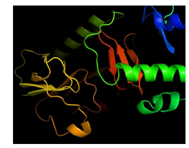
A. Schematic representation of the small molecule inhibitor profiling assay cascade.
B. Hit confirmation and routine design-make-test screening is performed by RapidFire Mass Spectrometry. Early exemplar STM10155 potency against SARS2, MERS and the closest human homologue to the viral methyltransferase.
C. Compounds too potent to accurately measure IC_{50}s in the RFMS assay are analyzed for direct binding by single cycle kinetic surface plasmon resonance (SCK-SPR). The binding data has an excellent correlation with the antiviral cell potency against SARS2 in A549-ACE2-TMPRSS2 cells.
D. STM10189 has single digit picomolar binding and a very slow off-rate by SCK-SPR.
E. Phylogenetic tree of 50 Coronaviridae. Storm compounds demonstrate antiviral cellular potency for different highlighted coronavirus subgenera: SARS2, MERS, HuCoV229E, HuCoVOC43, MHV and the emergent bat strain SHC14. No cytotoxicity up to 10µM (A549-ACE2-TMPRSS2, Huh7, MRC-5, DBT, VeroE6 & Vero81 cells).
F. Storm methyltransferase inhibitors are viral specific. The diagram shows profiling of STM10155 in a human methyltransferase panel (Reaction Biology and Storm). Data are displayed as percent enzyme activity relative to DMSO-treated controls.
G. Dose response graph of STM10189 inhibition of the cytopathic effect of SARS-CoV-2 human/USA/USA-WA1/2020 reporter infection in human airway epithelial cells grown at an air-liquid interface (HAE-ALI).

2) Optimized compounds are potent, stable and bioavailable

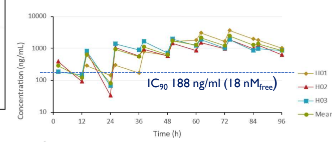
A Progress towards candidate



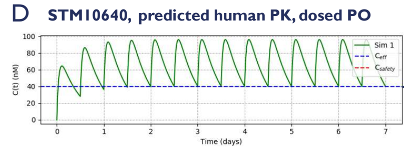
B Compound design is structurally enabled



C IP dosing to Ceff of STM10189



D STM10640, predicted human PK, dosed PO

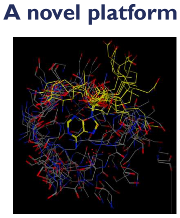


STM	MW	HBD	tPSA NO	logD
STM10640	478	2	110	3.2

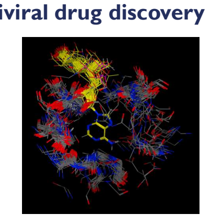
A. Graph illustrating the progress to date to make potent and metabolically stable analogues.
B. Crystal structures of Storm compounds bound to the target are used for structure-based design.
C. STM10189 is tolerated for BID intraperitoneal (IP) dosing at concentrations 5-fold the predicted efficacious antiviral concentration at Cmin (18nM free) in Syrian Golden Hamsters. A 4-day efficacy study is scheduled.
D. STM10640 has a predicted oral dose of 8.5 mg/kg BID in human to cover the unbound EC_{90} at Cmin. Recent potent analogues have improved cell potency, human hepatocyte stability and fraction absorbed in rodent in-vivo PK. Physicochemical properties are in good drug-like property space.

3) A novel platform for antiviral drug discovery

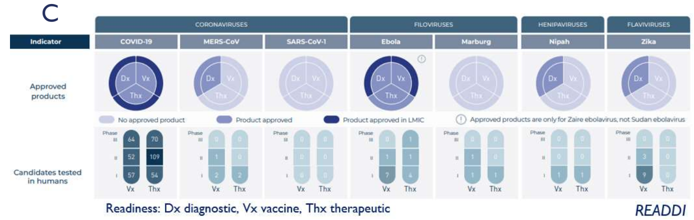
A



B



C



A. SAM/SAH, the methyltransferase cofactor/by-product, bound to a selection of non-viral human target proteins extracted from the PDB showing the diversity of conformations adopted in the proteins.
B. SAM/SAH bound to all the viral methyltransferases extracted from the PDB showing the similarity of the cofactor conformation and virus binding pocket, demonstrating the potential for pan-viral activity.
C. Most viruses transcribe their own enzymes for RNA capping. The pictogram indicates those viruses with pandemic potential which use similar methyltransferases and which have a significant unmet medical need from a lack of diagnostics, vaccines and treatments.

Summary

- STORM has developed novel, potent, first-in-class inhibitors of a coronavirus methyltransferase exhibiting high viral selectivity and 5-10 fold greater cell potency versus the oral standard of care.
- Structural and functional evidence indicates Storm viral methyltransferase inhibitors could be active against other viruses with pandemic potential and unmet medical need.
- *In-vitro* and *in-vivo* tool compounds are now available for further exploration of the immense potential of viral methyltransferase inhibitors for their unique dual mechanism of action, both anti-replicative activity and activation of the host innate immune response to the virus.

Harnessing the power of RNA modification